Award Number: DAMD17-00-1-0095

TITLE: Characterization of SIRPs in Prostate Cancer Cells

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REPORT DATE: March 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 074-0188*



| 1. AGENCY USE ONLY (Leave blank) | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | March 2003 | Annual (15 Feb 02 - 14 Feb 03) |

**4. TITLE AND SUBTITLE**
Characterization of SIRPs in Prostate Cancer Cells

**5. FUNDING NUMBERS**
DAMD17-00-1-0095

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**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**

**11. SUPPLEMENTARY NOTES**

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**12b. DISTRIBUTION CODE**

**13. ABSTRACT (Maximum 200 Words)**

Signal regulatory proteins (SIRPs include SIRPβ1, which activates cells, and SIRPα1, which inhibits the cellular response to several growth factors, and which regulates cell adhesion and spreading.

We demonstrated by PCR that 3 of 3 prostate cancer cell lines (PC-3, DU-145 and LNCaP) express transcripts for SIRPs. Under this contract, we generated a monoclonal antibody that recognizes both SIRPβ1 and SIRPα1, thereby confirming the expression of SIRPs on PC-3 cells and, to a lesser extent on DU-145 cells. The receptor could not be detected on LNCaP cells. We have since shown by PCR, Western blotting, and by surface staining that PC-3 and DU-145 cells express SIRPα1 but not SIRPβ. We find that they also express the tyrosine phosphatase, SHP-2, and that SHP-2 binds to SIRPα1 when it is phosphorylated, demonstrating that this pathway for the function of SIRPα1 is intact. We have created constructs of epitope-tagged SIRPα1, either intact or with mutations that would alter SHP-2 binding, in order to study its function in PC-3 cells.

We have also worked in particular on the characterization of the SIRPα1 protein in prostate cancer cells. Is there more than one form, due either to alternate splicing or to post-translational modification? These studies have proved challenging, but we expect to complete them, along with all of the objectives of the contract, over the coming year (no-cost extension).

**14. SUBJECT TERMS**
signaling, Prostate Cancer, Growth Factor, Signal Regulatory Protein SHPS-1

**15. NUMBER OF PAGES**
7

**16. PRICE CODE**

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| Unclassified | Unclassified | Unclassified | Unlimited |

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## INTRODUCTION

Our Studies are based on our identification by PCR of transcripts for signal regulator protein a1 (SIRPα1) in three prostate cancer cell lines, PC-3, LNCaP, and DU-145. We proposed six objectives:

1. Examine SIRP transcript size and expression level in different prostate cancer cell lines by Northern blotting.
2. Use RT-PCR to obtain and sequence full-length SIRP transcripts from the prostate cancer cell lines PC-3, DU-145, and LNCaP.
3. Use hybridization to screen cDNA libraries from these prostate cancer cell lines to identify additional SIRP cDNA clones.
4. Overexpress wild-type and mutated SIRPs in prostate cancer lines and, as a control, in NIH3T3 fibroblasts, to assess their effect on the cellular growth response to EGF. These will include studies of the effect of cross-linking SIRPs in cell growth. Mutational studies will include: for alpha SIRPs, mutate the cytoplasmic tyrosine to phenylalanine, for beta SIRPs, mutate the transmembrane lysine to alanine.
5. Assess the effect of EGF on the phosphorylation of SIRPs in prostate cancer cells and of associated proteins obtained by co-immunoprecipitation.
6. Produce monoclonal antibodies (mAbs) against SIRPs.

## BODY

Signal regulatory proteins (SIRPs, also known as SHPS-1, BIT, p84, and Myd-1) are normally expressed on certain hematopoietic cells and some brain cells (1-3). SIRPβ1 activates cells and is expressed on cells of monocyte/macrophage lineage. Its ligand is unknown. SIRPα1 inhibits the response of several cell types to growth factors (1), and it regulates integrin-mediated cell adhesion and spreading (4,5). Its ligand is CD47 (integrin associated protein)(6,7). SIRPβ1 and SIRPα1 are highly homologous in their extracellular portions, which include three immunoglobulin (Ig)-like domains (one V and two C domains). By alternative splicing, SIRPα1 can also be expressed with a single Ig-like (V) domain (8). Phosphorylated SIRPα1 binds SHP-2, a tyrosine phosphatase that is widely distributed (1). Thus, expression of SIRPs on tumor cells might be functional and could regulate the response to growth factors and/or the capacity of tumors to invade.

This report is for the third year of our studies. During the first year, we: (i) produced monoclonal antibodies to SIRPs (cross-reactive with both SIRPα1 and SIRPβ1), (ii) used the antibodies to confirm surface expression of SIRPs on PC-3 and DU-145 prostrate cancer cells (LNCaP did not stain with mAb, but had only low levels of transcripts by PCR), and (iii) stably overexpressed SIRPα1 and SIRPβ1 on PC-3 cells. These findings were published as an abstract for the Annual Meeting of the American Association for Cancer Research, March, 2001 (attached).

During the second year, we: (i) completed objective one by performing Northern blotting of RNA from PC-3, DU-145, and LNCaP cells using, as a probe, a PCR product covering most of the extracellular domain, which revealed (for PC-3 and DU-

145) a dominant band at ~3.5kb and a secondary band at ~2.2kb, similar to transcripts in the U373 glioblastoma cell line, which expresses SIRPα1 (unpublished); (ii) used specific PCR primers to demonstrate transcripts for SIRPα but not SIRPβ in PC-3 cells; (iii) conformed by Western blotting that SIRPα is expressed in PC-3 cells, (iv) performed hybridization screening of a PC-3 DNA library from Drs. Shutsung Liao and John Kokontis at the University of Chicago, which unfortunately led us to find that their subclone of PC-3 lacks SIRPα; (v), probed the NCBI human genome and the Celera human genome with each exon of SIRPβ1 and SIRPα1, by which we identified a only single gene for SIRPα within the SIRP family locus on chromosome 20, but also a second potential SIRPα gene on chromosome 22, where SIRPα is encoded as a single exon, evidently a retrotransposon (one of our PCR products correlates with this gene suggesting that genes both may be expressed in PC-3 cells), (vi) by the same methods, identified the known genes for SIRPβ as well as several loci that may encode other SIRPβ proteins (but as confirmed in this year's work, these are not expressed in the prostate cancer cells), (vii) used PC-3 cells treated with pervanadate (to increase tyrosine phosphorylation of all proteins) to demonstrate that PC-3 cells express SHP-2 tyrosine phosphatase, (viii) demonstrated association of SIRPα with SHP-2 in PC-3 prostate cancer cells, (ix) initiated studies using protein deglycosylation to confirm the size of SIRPα in prostate cancer cells.

During the third year, we spent much of our effort on the characterization of intact and deglycosylated SIRPα protein in PC-3 cells. Although we are experienced in these area, we went through a prolonged period in which we obtained inconsistent results in these studies. We believe we have resolved these issues with the finding that the PC-3 cells express some full-length SIRPα protein, but that there may be an additional, smaller form. Because of these problems, we requested and received a no-cost extension, and we expect to completer our studies within this time.

Additional results obtained during the third year include (i) confirmation that SIRPβ is not expressed on the prostate cancer cell lines by using a monoclonal antibody that recognizes SIRPβ1 but not SIRPα1; (ii) construction of transcripts encoding SIRPα1 mutated at the cytoplasmic tyrosine required for the recruitment of SHP-2; (iii) production of additional monoclonal anti-SIRP antibodies.

## KEY RESEARCH ACCOMPLISHMENTS

### *Year 1*

1. The production of monoclonal antibodies to SIRPs
2. The use of monoclonal antibodies to confirm the expression of SIRPs on prostate cancer cells.
3. Stable overexpression of SIRPα1 and of SIRPβ1 in PC-3 cells.

### *Year 2*

1. Confirmation of SIRP transcripts in prostate cancer cells by Northern blotting.
2. Confirmation of SIRPα1 transcripts in PC-3 cells by PCR (no evidence for SIRPβ).
3. Conformation by Western blotting that SIRPα is expressed in PC-3 cells
4. Demonstration that PC-3 cells express the SIRP substrate SHP-2
5. Demonstration in PC-3 cells of the interaction of SIRP with SHP-2.

### *Year 3*

1. Resolution of SIRPα protein size, expressed in prostate cancer cells in both glycosylated and deglycosylated forms (this work is still in progress).
2. Demonstration by flow cytometry that prostate cancer cells do not express SIRPβ.
3. Construction of mutant SIRPα1, lacking the cytoplasmic tyrosine required for the recruitment of SHP-2.

## REPORTABLE OUTCOMES

PC-3 cells express SIRPα, and phosphorylation of this receptor leads to its association with the tyrosine phosphatase, SHP-2. We wish to resolve the exact form of SIRPα before we report this.

## CONCLUSIONS

Our studies have confirmed the hypothesis that prostate cancer cell lines express transcripts for SIRPα and that SIRPα is expressed on the cell surface. Further, they express SHP-2, and this phosphatase associates with phosphorylated SIRPα in PC-3 prostate cancer cells, supporting the hypothesis that this receptor is functional. Studies with Western blotting suggest that PC-3 cells may express both full-length SIRPα and a smaller form, as yet uncharacterized.

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